

SUPPORTING INFORMATION

Kratom alkaloids as probes for opioid receptor function: Pharmacological characterization of minor indole and oxindole alkaloids from kratom

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#deceased

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HPLC method to determine purity

Instrument: Agilent 1200 Series HPLC

Column: Higgins Analytical CLYPEUS C18 column (5 μ m, 150 \times 4.6 mm),

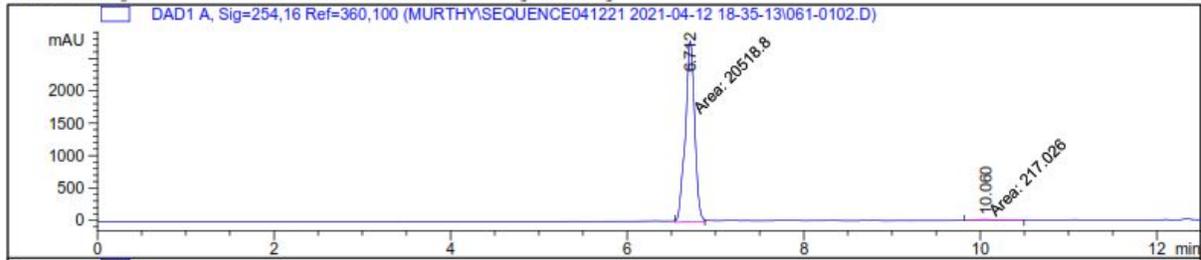
Method: Gradient elution program–(acetonitrile/water 5/95/95/5, 0.1% TFA), flow rate=0.65 ml/min.

HPLC profile of compound 5

Data File C:\CHEM32\1\DATA\MURTHY\SEQUENCE041221 2021-04-12 18-35-13\061-0102.D
 Sample Name: Comp5

```

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Acq. Operator   : Ankita                      Seq. Line :    1
Acq. Instrument : Instrument 1                  Location  : Vial 61
Injection Date  : 4/12/2021 6:49:52 PM      Inj       :    2
                                           Inj Volume: 10 µl
Acq. Method     : C:\Chem32\1\DATA\MURTHY\SEQUENCE041221 2021-04-12 18-35-13\MURTHY100120-1.M
Last changed    : 10/29/2020 10:54:32 PM by Murthy
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Last changed    : 10/29/2020 10:54:32 PM by Murthy
  
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Area Percent Report

```

Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
Use Multiplier & Dilution Factor with ISTDs
  
```

Signal 1: DAD1 A, Sig=254,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.712	MM	0.1223	2.05188e4	2797.00708	98.9534
2	10.060	MM	0.2763	217.02577	13.08905	1.0466

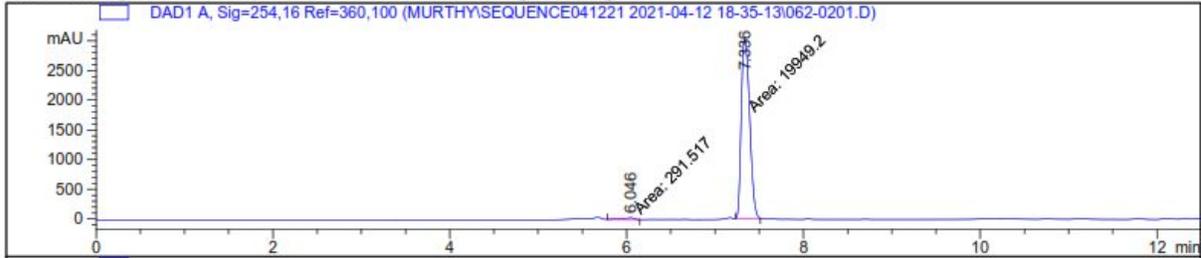
Totals : 2.07358e4 2810.09613

Instrument 1 4/13/2021 1:15:32 PM Ankita

HPLC profile of compound 6

Data File C:\CHEM32\1\DATA\MURTHY\SEQUENCE041221 2021-04-12 18-35-13\062-0201.D
Sample Name: Comp6

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Acq. Operator   : Ankita                               Seq. Line :    2
Acq. Instrument : Instrument 1                         Location  : Vial 62
Injection Date  : 4/12/2021 7:04:18 PM                Inj       :    1
                                                    Inj Volume: 10 µl
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Last changed    : 10/29/2020 10:54:32 PM by Murthy
Analysis Method : C:\CHEM32\1\DATA\MURTHY\SEQUENCE041221 2021-04-12 18-35-13\MURTHY100120-1.M
Last changed    : 10/29/2020 10:54:32 PM by Murthy
=====
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Area Percent Report

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Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
Use Multiplier & Dilution Factor with ISTDs
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Signal 1: DAD1 A, Sig=254,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.046	MM	0.1529	291.51669	31.77309	1.4402
2	7.336	MM	0.1097	1.99492e4	3031.75049	98.5598

Totals : 2.02407e4 3063.52358

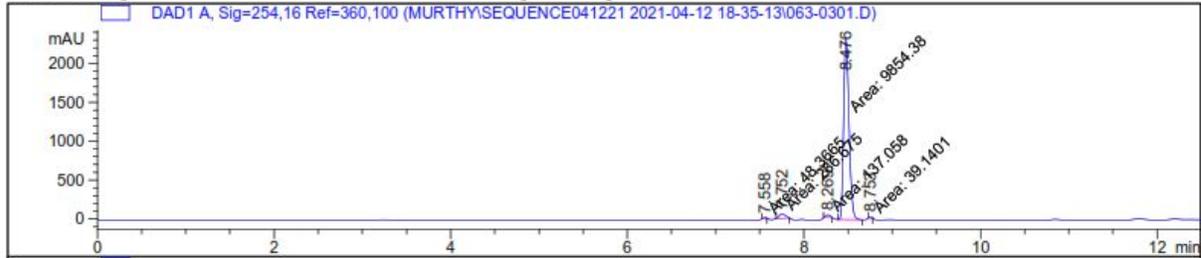
Instrument 1 4/13/2021 1:17:22 PM Ankita

HPLC profile of compound 7

Data File C:\CHEM32\1\DATA\MURTHY\SEQUENCE041221 2021-04-12 18-35-13\063-0301.D
 Sample Name: Comp7A

```

=====
Acq. Operator   : Ankita                               Seq. Line :    3
Acq. Instrument : Instrument 1                          Location  : Vial 63
Injection Date  : 4/12/2021 7:18:44 PM                Inj       :    1
                                                    Inj Volume: 10 µl
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Last changed   : 10/29/2020 10:54:32 PM by Murthy
Analysis Method: C:\CHEM32\1\DATA\MURTHY\SEQUENCE041221 2021-04-12 18-35-13\MURTHY100120-1.M
Last changed   : 10/29/2020 10:54:32 PM by Murthy
  
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Area Percent Report

```

=====
Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
Use Multiplier & Dilution Factor with ISTDs
  
```

Signal 1: DAD1 A, Sig=254,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.558	MM T	0.0422	48.36651	19.08728	0.4666
2	7.752	MM T	0.0801	286.67545	59.62195	2.7656
3	8.269	MM T	0.0642	137.05786	35.56246	1.3222
4	8.476	MM T	0.0707	9854.37988	2321.70752	95.0679

Data File C:\CHEM32\1\DATA\MURTHY\SEQUENCE041221 2021-04-12 18-35-13\063-0301.D
 Sample Name: Comp7A

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
5	8.753	MM T	0.0329	39.14009	19.79964	0.3776

Totals : 1.03656e4 2455.77885

Instrument 1 4/13/2021 1:23:18 PM Ankita

Table S1. Functional binding data of the compounds at opioid receptors.

Receptors	Compounds	G protein activation (BRET) assay		Arrestin recruitment (BRET) assay		Figure
		EC ₅₀ nM (n = 3) ± SEM	Emax% ± SEM	EC ₅₀ nM (n = 3) ± SEM	Emax% ± SEM	
hMOR	Isopaynantheine Corynantheidine	1259 (5.9 ± 0.12)	156.91 ± 13.49	1276.4 (5.89 ± 0.14)	106 ± 8.67	6C-D 2A-B
hMOR		67.21 (7.17 ± 0.29)	37.22 ± 4.3	nd	<20	
hMOR	Diprenorphine DAMGO	4.28 (8.37 ± 0.05)	100 ± 2.90	0.43 (9.37 ± 0.10)	100 ± 2.47	6C-D 2A-B
hMOR		3.56 (8.45 ± 0.13)	100 ± 4.17	172.89 (6.76 ± 0.08)	100 ± 3.48	
hKOR	Corynantheidine	nd	<20	nd	<20	2C-D
hKOR	U50488H	2.8 (8.55 ± 0.21)	100 ± 5.89	229.54 (6.64 ± 0.11)	100 ± 4.63	2C-D
hDOR	Corynantheidine	nd	<20	nd	<20	2E-F
hDOR	DPDPE	1.23 (8.91 ± 0.28)	100 ± 11.69	12.28 (7.91 ± 0.12)	100 ± 4.08	2E-F
hMOR	Corynoxine	1630 (5.79 ± 0.09)	96.54 ± 4.69	nd	<20	3A-B
hMOR	DAMGO	28.12 (7.55 ± 0.08)	100 ± 2.78	90.6 (7.04 ± 0.07)	100 ± 2.29	3A-B
hKOR	Corynoxine	nd	<20	nd	<20	3C-D
hKOR	U50488H	6.93 (8.16 ± 0.06)	100 ± 1.61	48.95 (7.31 ± 0.04)	100 ± 1.47	3C-D
hDOR	Corynoxine	nd	<20	nd	<20	3E-F
hDOR	DPDPE	3.65 (8.43 ± 0.37)	100 ± 14.51	17.69 (7.75 ± 0.17)	100 ± 5.8	3E-F
hMOR	Mitracilicatine	23.69 (7.62 ± 0.16)	50.75 ± 3.22	nd	<20	5A-B
hMOR	DAMGO	3.56 (8.45 ± 0.13)	100 ± 4.17	90.6 (7.04 ± 0.07)	100 ± 2.29	5A-B
hKOR	Mitracilicatine	269.19 (6.57 ± 0.06)	97.6 ± 2.71	383.12 (6.42 ± 0.06)	104.13 ± 2.57	5C-D
hKOR	U50488H	6.93 (8.16 ± 0.06)	100 ± 1.61	48.95 (7.31 ± 0.04)	100 ± 1.47	5C-D
hDOR	Mitracilicatine	nd	<20	nd	<20	5E-F
hDOR	DPDPE	3.65 (8.43 ± 0.37)	100 ± 14.54	17.69 (7.75 ± 0.17)	100 ± 5.8	5E-F
hMOR	Isopaynantheine	nd	<20	nd	<20	6A-B
hMOR	DAMGO	31.27 (7.50 ± 0.03)	100 ± 1.51	81.62 (7.09 ± 0.06)	100 ± 2.55	6A-B
hKOR	Isopaynantheine	560.4 (6.25 ± 0.07)	80.51 ± 2.93	599.29 (6.22 ± 0.43)	<20	6E-F
hKOR	U50488H	7.03 (8.15 ± 0.04)	100 ± 1.58	128.31 (6.89 ± 0.10)	100 ± 4.12	6E-F
hDOR	Isopaynantheine	nd	<20	nd	<20	6G-H
hDOR	SNC80 (Gi-1) DPDPE(arrestin)	13.37 (7.87 ± 0.05)	100 ± 1.89	7.76 (8.11 ± 0.05)	100 ± 1.81	6G-H

Data from both Gi-1 activation and arrestin assays using human opioid receptors were normalized to E_{\max} of the corresponding controls, DAMGO, U50,488H, DPDPE and Diprenorphine, where, the dose response curves were fit using a three-parameter logistic equation in GraphPad Prism and the data are presented as mean EC_{50} ($pEC_{50} \pm SEM$) for assays run in triplicate.